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NEUROHISTOLOGICAL INVESTIGATIONS ON GENERAL OXYGEN DEFICIENCY OF THE BRAIN

The Morphological Behavior of the Ganglion Cells After Generalized Acute and Subacute Hypoxia

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THE MORPHOLOGICAL BEHAVIOR OF THE GANGLION CELLS AFTER GENERALIZED ACUTE AND SUBACUTE HYPOXIA

In studies on astrocytes carried out both in human postmortem material and, in collaboration with Noell,* in the brains of cats, we found that the type of transition from the normal oxygen supply of the tissue to the anoxia of death has a great influence on the postmortem behavior of the cells. The main finding was as follows: If death was preceded by a hypoxic phase lasting for a period of seconds to about 60 minutes, a disintegration of astrocytes, which Cajal called *clasmatodendrosis*, developed immediately after death. If the hypoxic phase lasted more than an hour, the astrocytes maintained their form for a period of time after death.

In the following, equivalent studies on ganglion cells will be reported. They were performed on animal brains (cats) as well as on human autopsy material. The findings in the animal experiments will be described first, followed by those made in human material.

EXPERIMENTAL

1. Methods

The brains of 55 full-grown cats were examined. Each animal was given one exposure to oxygen deficiency in which it died. The duration of the exposure varied from seconds to about 6 hours. In 7 cats the oxygen supply to the brain was cut off instantaneously by decapitation. In 32 cats oxygen deficiency was produced by lowering the barometric pressure (high altitude chamber); in all others, by exposing them to carbon monoxide or nitrogen mixtures. After a generally rapid increase of hypoxia, the animals were kept below the subcritical to critical levels for varying periods of time with the effect that in the prolonged experiments the clinical picture was very similar to that of agony. In some animals from the experimental series performed in collaboration with Noell, the intensity of the oxygen deficiency was controlled electroencephalographically, as reported in the pertinent paper. In most animals the degree of oxygen deficiency was estimated with the help of the clinical picture as described for the cat in its various stages by Altmann and Schubothe (1). The behavior of the coarse motor functions and of respiration was of special importance. After reaching a certain degree of hypoxia, the animal showed strong, uncoordinated motor excitation which looked rather alarming. It jumped up, reared, twisted, and fell back again as if paretic. In contrast with its normal habits of cleanliness, the cat seemed not to care if it was soiled with feces or urine. It showed panting respiration, 150 to 200 breaths per minute. This excitation phase, which in view of the strong individual variations in the animal's resistance might set in as low as 24,000 feet in one animal and as high as 30,000 feet in another, was succeeded by a phase of motor inactivity as hypoxia increased. The animal lay stretched out and seemed to experience severe clouding of consciousness. Rapid respiration continued as a rule, although interrupted by individual deep breaths with succeeding brief pauses. Occasionally, the animal stood up once more for a few seconds, as if in a state of delirium, and collapsed again. From the clinical standpoint this phase might be designated as subcritical. When the hypoxia was increased but little or kept constant for prolonged periods of time, this phase went over into the critical phase, which was characterized by a considerable reductio of the respiratory rate (10 to 20 breaths per minute). The animal lay completely quiet. Occasionally, one could observe a fine trembling or some coarse twitches of one of the legs or of the mouth. These sometimes developed into a generalized epileptic attack which was succeeded by fatal respiratory paralysis. Yet, even without such an attack, the respiration, as in the case of human agony, might become slower and more superficial all the time, and in the end cease entirely.

* Lindenberg, R., and W.K. Noell, "The Effect of Hypoxia (In Vita) on the Postmortem Changes of the Astrocytes." (To be published)

After death the brains of a few animals were cooled to room temperature; some others were kept at 32° C. under sterile, humid conditions; most of them were kept at 37° C. under the same conditions. At certain time intervals, parts of the brain—in general one-half hemisphere—were excised and fixed. Since after 12 hours in the incubator putrefactive bacteria usually started to proliferate, some brains were kept in a body-warm saline solution to which some streptomycin and penicillin had been added. In this way it was possible to keep brain sections sterile in the incubator for as long as 72 hours. For fixation the brain sections were placed in 10 percent neutral formalin for one day. Ther the sections were washed, further hardened in 96 percent alcohol, and finally embedded in parlodion in the usual way. The ganglion cells were stained with cresyl violet and thionine.

II. The equivalent picture

In order to obtain a good equivalent picture, for the purpose of comparing the cells, the brain of one cat was fixed in 10 percent neutral formalin immediately after decapitation and then treated in the same way as the other brain sections. To enable the reader himself to compare the observed cell changes with the equivalent picture, four of the numerous types of ganglion cells are shown in figure 1.

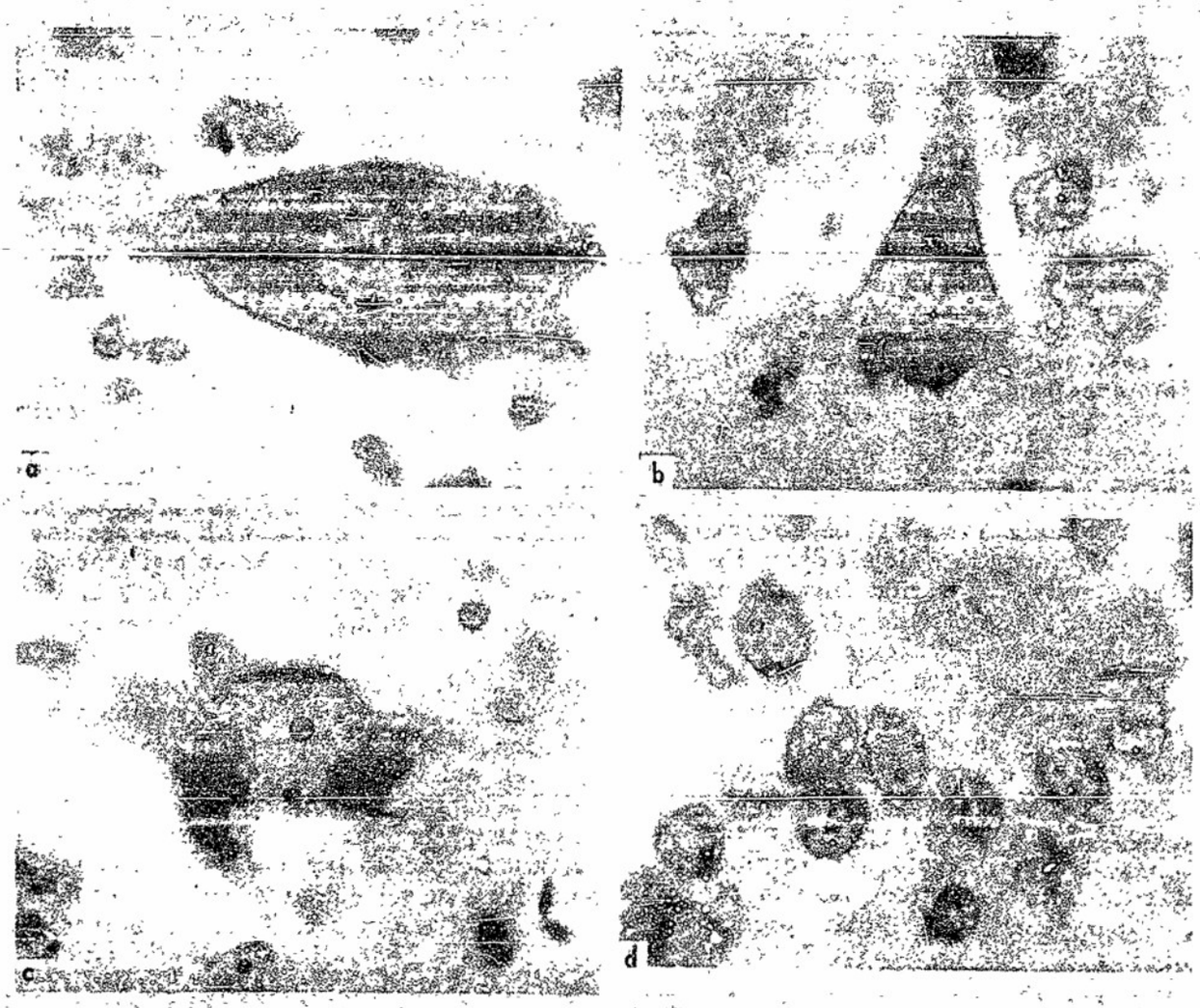

FIGURE 1

"Equivalent pictures" to the living ganglion cells of a cat. The cells have been fixed with formaldehyde at the moment of decapitation.

These include the large somatochrome pyramidal cell of the third and, generally, fifth layer of the cerebral cortex (figure 1a). It has a centrally located light nucleus with a well-marked nucleolus and scanty linin structure. In the cell body we find a clear arrangement of Nissl bodies near the nucleus and toward the base of the cell, where they are small and somewhat irregular in form; in the periphery of the cell and in the dendrites, they are large, elongated, and in rather parallel arrangement with ample unstained cytoplasm in between. Then, there are the medium-sized and small pyramidal cells of the cortex, which also belong to the somatochrome type. The nuclei are in general only a little smaller than those of the large cells. Their position is mostly somewhat eccentric. The Nissl bodies are composed, on the whole, of finer granules, especially in the vicinity of the nucleus, and arranged more densely. Toward the dendrites and, in particular, the apical dendrite, they are again elongated and separated farther from one another by unstained strands of protoplasm (figure 1b). Another somatochrome type is represented by the cells of the thalamus, which have a relatively good arrangement of the Nissl bodies (figure 1c). They are irregular in form and are connected more like a net. They are again finer in the vicinity of the nucleus and coarser toward the periphery of the cell. Most nuclei are large and light, having a well-marked nucleolus. In contrast with these cells are the cells of the second cortical layer with their meager cell bodies. Their nuclei are most distinct. Therefore, Nissl (2, 3) designates this cell type as *karyochrome*. The nuclei are somewhat smaller than those described above, some of them showing fine folds. The linin structure is generally more distinct, the nucleolus relatively small. The meager cell body contains but little, usually dustlike Nissl substance in the immediate vicinity of the nucleus, or in one of the narrow dendrites (figure 1d).

III. Findings

Before the histological details are discussed, one observation that was made during the gross examination of the brains should be reported. The brains of those animals which had died within a few minutes showed a certain increase in volume; this was not the case in the animals that had been exposed to hypoxia for hours. As a rule, the former felt firm, as if stretched tightly; the latter had the usual medium compact consistency. When the brains had been kept in the saline solution for 24 hours and longer, the former became continuously softer, and it was difficult to cut them in the unfixed state without squashing them. The latter, however, were considerably more compact and could be cut without difficulty. Such softness of the tissue is very similar to the consistency of fresh softenings. This will be referred to later.

The description of the histological findings will be started with the changes found in the decapitated animals whose brains had been kept at 37° C. The first change could be observed after about 30 minutes. The larger ganglion cells showed a mild swelling of the cell body. The protoplasm surrounding the nucleus became lighter. The Nissl substance in this section started to break up (tigrolysis). The coarse Nissl granules were pressed toward the periphery of the cell body. Sometimes the nucleus was also displaced sideways. The nucleus may have been especially pale or showed intensified marking of the linin structure. Figures 2a, b, and c show this alteration in the somatochrome cell types demonstrated in figure 1. In the small karyochrome cell types, a similar swelling could be found; usually, however, small circular transparencies of the protoplasm had already developed, surrounded by darker, stained protoplasm (figure 2d). By the term *swelling* we do not mean Nissl's "acute change in ganglion cells" which Spielmeyer (4) called *acute swelling*, and which was not observed in our material. The swelling we are concerned with is obviously another likewise acute process which—to make a parallel with Spielmeyer's *simple shrinkage*—we term *simple swelling*. The reason for this designation is that, aside from the pictures of simple swelling, we see simple shrinkage in some of the larger pyramidal cells or in the cells of small cortical sections. Gildea and Cobb (5) observed these pictures in their experiments, likewise very shortly after death. We found them usually in the region of the gyral crests. Since Scharrer (6) was able to produce, by local pressure, such early pictures of shrinkage locally confined in the body-warm brain, we are inclined to assume that in our cases they were also caused by pressure exerted when the brains were taken out. In such a case, changes or even lesions of the cell wall obviously developed, resulting in loss of fluid and shrinkage of the cell. With its

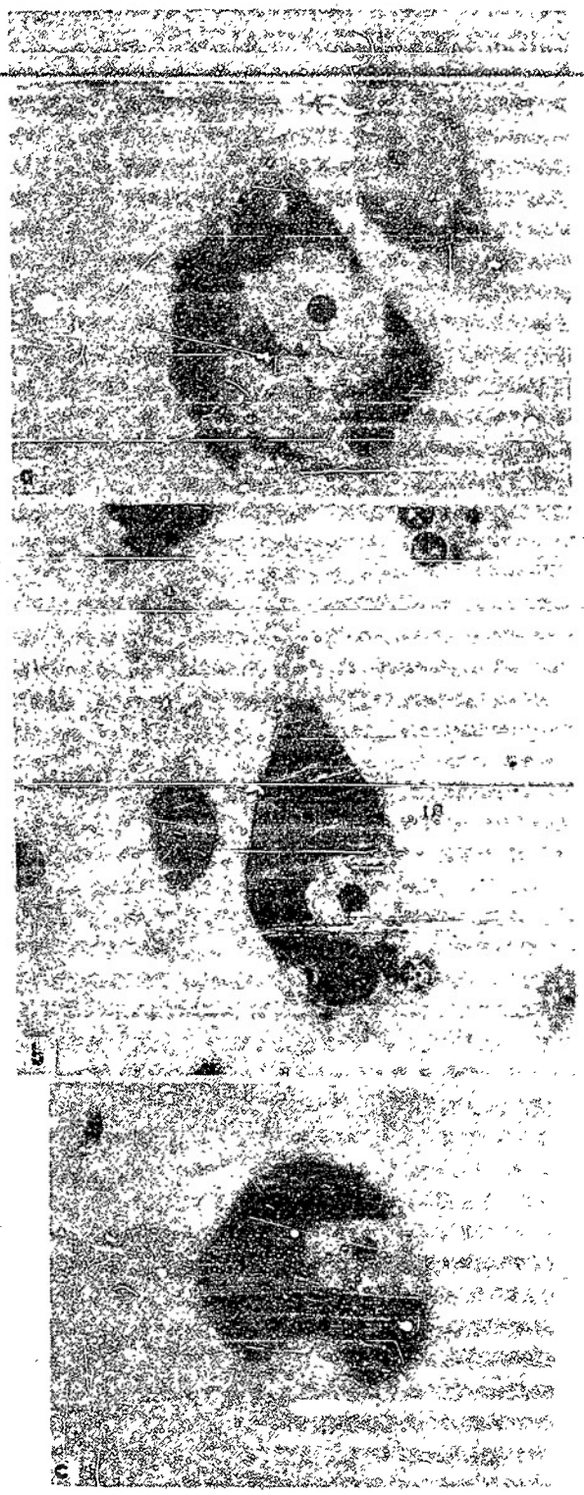

FIGURE 2

"Simple swelling" of somatochrome cell types of a cat developed within 30 minutes after decapitation. The unfixed tissue has been kept at 37° C.

wall intact it would have absorbed more fluid, thus leading to the picture of simple swelling.

After 6 hours in the incubator, the histological picture of a brain section had undergone considerable changes of different types. Two main types of changes were found which occur, more or less combined, in many cells. The one type demonstrated the onset of a homogenizing process. The Nissl substance, except for a few very small particles, had disintegrated; here and there dustlike remnants could be recognized. The cell body, generally still swollen, had acquired a fine-granular appearance, as if its protoplasm had coagulated. It could stain dark or light. The nucleus could be distinctly visible or it could be smaller and dark-stained; or it could have lost its membrane, so that its former position could be recognized only by a light spot containing the nucleolus and some particles of the linin structure. Later stages of this change are demonstrated in figure 5b. As to the second type of changes, minute vacuoles developed in the unstained protoplasm tracts between the Nissl bodies. These vacuoles may have increased in size and, after 6 hours, filled the entire cell body (figure 3a). The nucleus became small, dark-stained, angular, and often did not show up well against the network of the cytoplasm. Both processes were combined in the large pyramidal cell shown in figure 3b. The swelling of the cell is distinctly visible. Such pictures were found quite often, e.g., in the Purkinje cells of the cerebellum. It was noticed that the incidence of either type of change varied greatly with the individual animals. In one animal, for instance, the vacuolar alteration was predominant; in another, the homogenizing process. We checked whether this might be due to different preparation of the material, but found no positive evidence in this direction. The nutritive state of the cell at the instant of oxygen deprivation might possibly have some bearing in this respect.

In blocks kept unfixed in the incubator for 12 hours, the described changes were even more pronounced. The cells which had undergone the homogenizing process had, as a rule, dark, shrunken nuclei; their protoplasm showed poorer and poorer response to staining (figure 4a). At this time, obviously, vacuoles no longer developed in such cells. The cells which had been subjected to severe vacuolization showed still greater changes. They

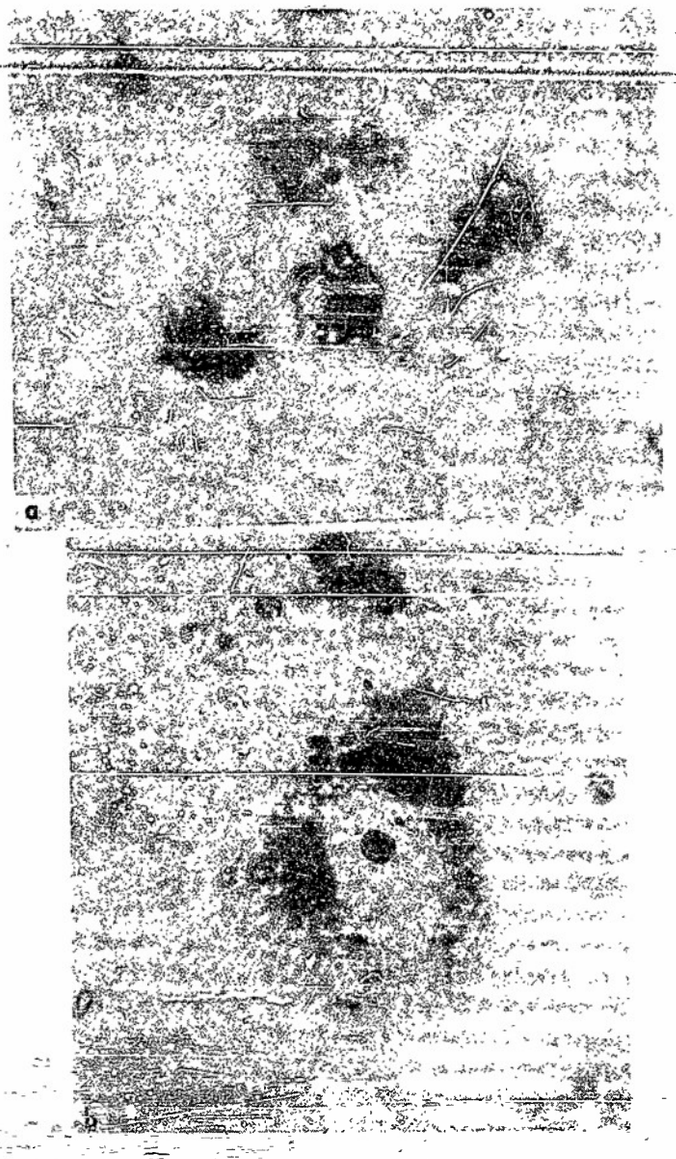

FIGURE 3.

Homogenization and vacuolization of ganglion cells of a cat 6 hours after decapitation. The unfixed tissue has been kept at 37° C.

may have acquried an absolutely foamy structure, in which case the cell limits were no longer clearly visible (figure 4b). The membranes of the vacuoles were composed of dark-staining protoplasmic trabeculae which formed a more or less dense network in which the completely shrunken nucleus could be recognized only by the resistant nucleolus. In some of the cells which showed both homogenization and peripheral vacuolization, one could see that the vacuoles contacted and dilated the pericellular space (figure 4c). The more solid part of the cell body often shrunk (figure 4d). This likewise represents a "simple shrinkage" although of a cell already greatly changed. In contrast to the early pictures of shrinkage, the Nissl structure in the cell was no longer visible. The protoplasm had in general a granular appearance. A greater number of the small pyramidal cells now showed pictures of shrinkage with winding apical dendrites.

After a period of 12 hours, the changes proceeded at a slower rate than before. After 48 hours, the picture of Spielmeyer's "ischemic changes in ganglion cells" had developed in most cells (figure 5a). As a rule, the nuclei were shrunken, often triangular in shape. Their outlines were frequently rather indistinct. In a few cells the nucleus would be relatively intact; in others it would be just about disintegrated. The protoplasm was homogeneous and pale and would show either signs of the previous swelling or various degrees of shrinkage. In the cell body, which looked as if it were coagulated, one could occasionally recognize a few dustlike particles of the former Nissl bodies (figure 5b). The Purkinje cells of the cerebellum showed the same changes (figure 5c). This picture presents very clearly the beginning regressive changes in the glial nuclei, which appeared about that time. The cells which had shown strong vacuolization were disintegrated to a great extent. As a rule, one could find nothing but a dark spot, the remainder of the nucleus surrounded by a shadowy remnant of the cell body. The cells which had shown coagulation phenomena in the center and vacuolization in the periphery were distinguished somewhat more clearly (figure 5d). Sometimes, one could see dark-stained particles in the periphery, which looked like incipient incrustations. In those parts of the cortex which were in direct contact with the surrounding saline solution well-marked "incrustations" of either coarse- or fine-granular structure were found, especially in the small pyramidal cells (figures 6a and 6b).

Up to 72 hours, the number of cell shadows increased. The larger cells alone showed some response to staining. In the smaller cells only the small dark nuclei could usually be recognized (figure 7). The regressive changes in the

glia, including even complete disintegration of the nucleus, could be seen everywhere.

This entire process was the same, though retarded, when the brain was kept at 18° C. Only after 12 hours did the first signs of homogenization and vacuolization appear. At a storage temperature of 32° C., the difference in the rate of this process was correspondingly less. Disintegration did not proceed at the same rate in all cells. Some somatochrome cell types in the deeper brain stem, particularly in the medulla oblongata, showed merely a swelling even as late as 12 hours after death in tissue stored at 37° C. The Nissl bodies stained paler but were still easily distinguished (figure 8). Occasionally, the nuclei were displaced eccentrically, and the branching point of the axonal dendrite, which was free of Nissl bodies, was expecially well marked. Some pictures were very similar to the initial stage of the retrograde cell change.

At a storage temperature of 37° C. the described changes developed, with little variation,

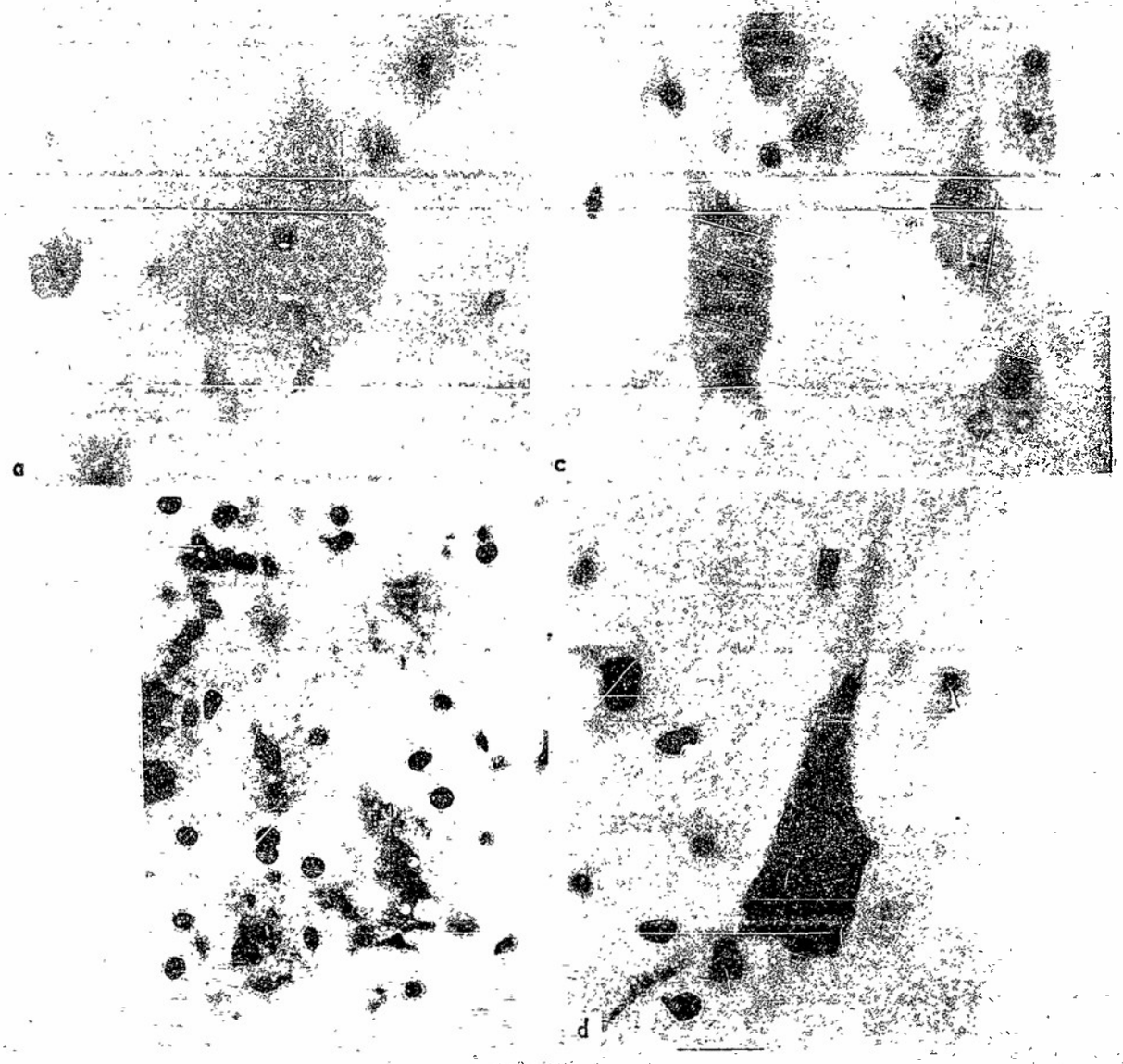

FIGURE 4

Further developed changes of ganglion cells within 12 hours after decapitation. a-c, Vacuolization and homogenization of the cells. d, Shrinkage of a homogenized ganglion cell with dilatation of the pericellular space at the left. The tissue has been kept at 37° C. before fixation.

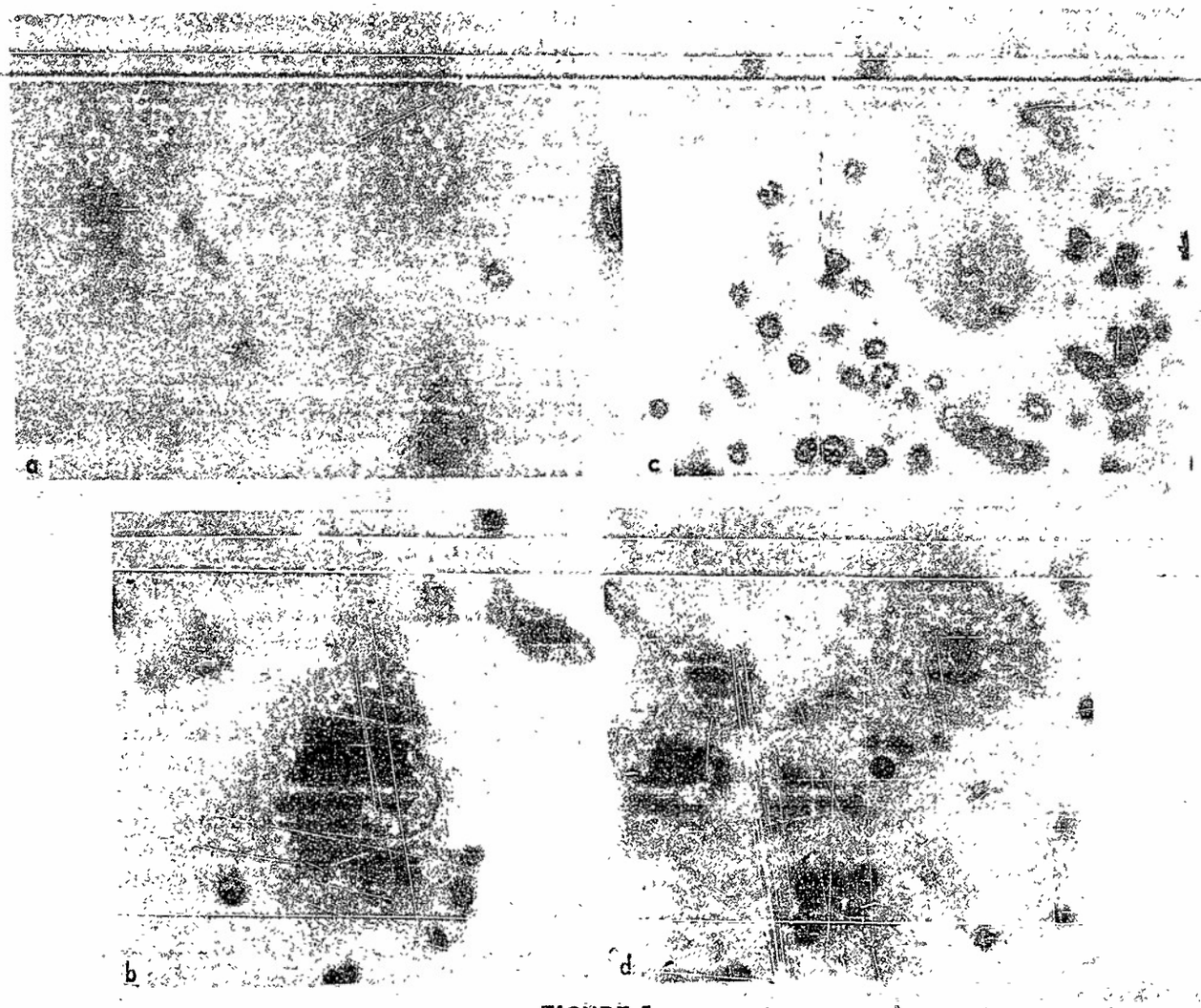

FIGURE 5

Cell changes developed within 48 to 72 hours after decapitation. Ischemic neuronal disease (a) and homogenization (b) of pyramidal cells, homogenization of a Purkinje cell (c), and further degeneration of partly vacuolized cells of the brain stem (d). The tissue has been kept at 37° C. before fixation.

also when death occurred after a critical oxygen deficiency up to about 10 minutes' duration. In such cases the increase of oxygen deficiency up to this degree was always very steep; the type of oxygen deficiency was irrelevant. If oxygen deficiency, after a similar steep increase, lasted about 20 minutes, swelling associated with tigrolysis likewise developed. As a rule, this swelling was not as complete as in the other cases. Figure 9a shows cells which had been fixed 3 hours after death. The cell body has a rather flaky appearance. The darker parts correspond to the Nissl bodies undergoing disintegration; the lighter ones, to the unstained tracts. Toward the apical dendrite, the remnants of some Nissl bodies are visible. The periphery of the cell body is transparent, yet without vacuoles. The nuclei are but little decreased in size. After 12 hours, the cell body still had the same flaky appearance (figure 9b). The nuclei had not changed much either.

If oxygen deficiency lasted longer than about 30 minutes, shortly after death the swelling could hardly be found. In fact, one had the impression that there were even a mild shrinkage and pyknomorphia (Nissl (2, 3)). In most cells

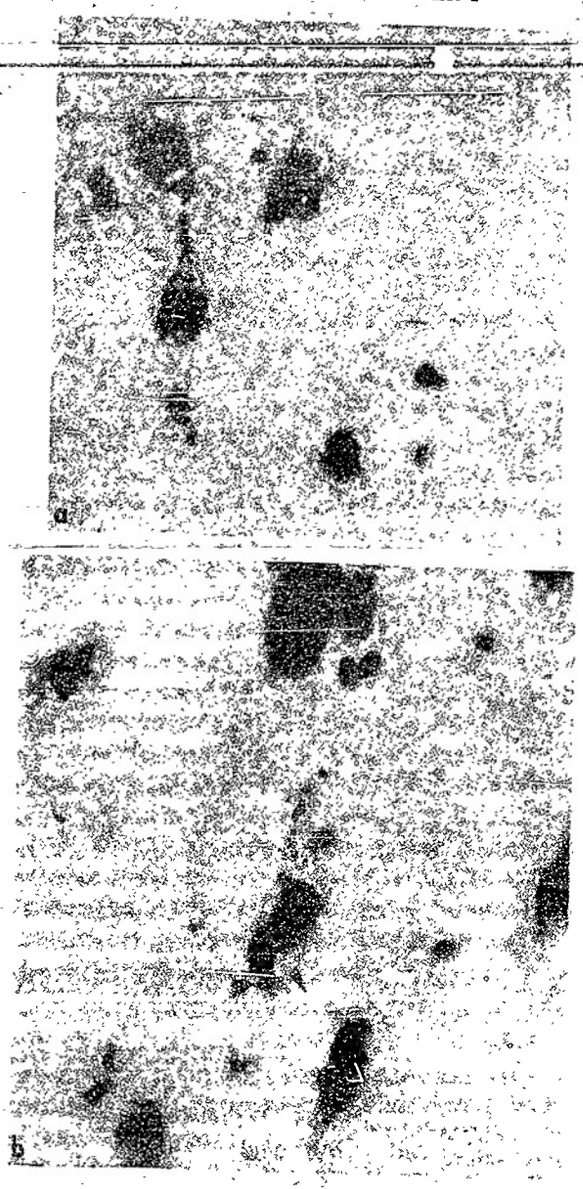

FIGURE 6

Pyramidal cells with incrustation of the "Golgi nets" fixed 48 (a) and 72 (b) hours after decapitation. The tissue has been kept at 37° C. before fixation.

this picture hardly changed during the 12 hours after death. In others, mostly the small pyramidal cells, a certain degree of tigrolysis had developed with flaky structure of the protoplasm. Figures 10a and 10b originate from such a case in which fixation was made 12 hours after death. Only in few cells, like those of the pallidum, was the Nissl structure more distinct. Most cells of the thalamus showed an increased degree of pyknomorphia; their nuclei were usually dark and reduced in size.

The longer the duration of oxygen deficiency, the smaller the number of changes in the cells. With a duration of about 60 minutes the cell pictures in the brain sections fixed after 30 minutes did not differ from the equivalent pictures shown in figure 1. Neither did the cells change when the brain sections were kept unfixed in the incubator at 37° C. for 12 hours. Figures 11a to 11d give some examples of such cells. Figure 11b shows that even the presence of a few putrefactive bacteria does not influence the cell structure. Only when the bacteria occurred in larger numbers did the cell structure become blurred; and the cell body and nucleus stained darker, showing a dirty, greyish-blue tinge, which differed somewhat from that in pyknomorphia or in beginning homogenization. This may be called pseudo-homogenization. Even the small cells of the second cortical layer showed no pathological changes. Nor did they undergo any noticeable alteration when the brain was kept in a saline solution at the same temperature for 18 hours (figures 12a and 12b). After 48 hours in the incubator, the cells showed some decrease in stainability. They looked a little paler; their structure, however, was principally unchanged (figure 13). If the brain was left in the solution for a still longer time, the paleness of the cells, especially in the cortex, increased; whereas the smaller cells of the thalamus, for instance, stained darker, showing a blurred marking of their fine-granular Nissl structure.

In a few of these cases of subacute hypoxia, a small focus was found in which the cells had undergone the same postmortem changes as in decapitated animals. Since we interpret these local softenings as a secondary effect of generalized hypoxia which is due to a local disturbance of vascular functions or to gas embolism, we do not want to discuss them in greater detail. We are interested only in the primary effect of generalized oxygen deficiency on the ganglion cells.

IV. Summary of experimental findings

In all cases in which the brains, within a period ranging from seconds to about 10 minutes, passed from the state of normal oxygen supply through a phase of severe oxygen deficiency into a state of anoxia, severe regressive

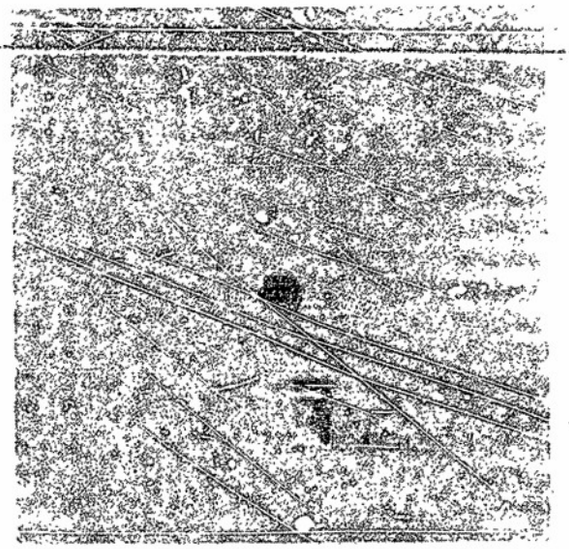

FIGURE 7

Shadow of a ganglion cell which has been fixed 48 hours after decapitation. The tissue has been kept at 37° C.

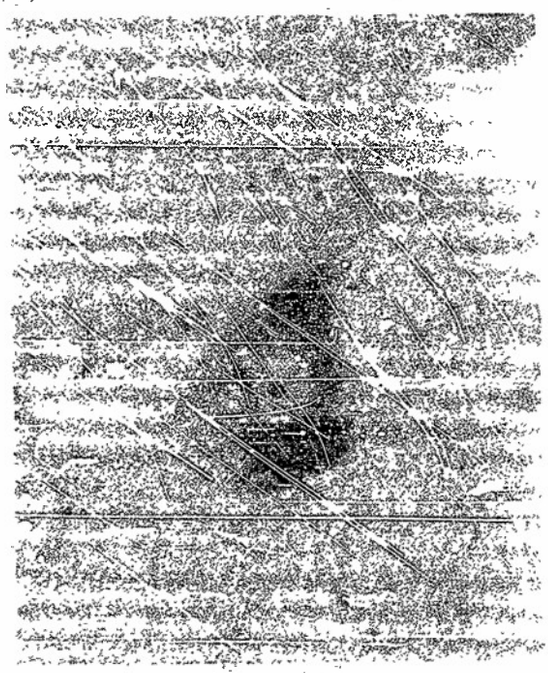

FIGURE 8

Swollen, somatochrome ganglion cell of the medulla oblongata of a cat 12 hours after decapitation. The tissue has been kept at 37° C.

changes developed, in the cells of the unfixed tissue kept at 37° C. These changes started with a simple swelling and tigrolysis of the cell, which may appear as early as 30 minutes after death. In the following 3 to 6 hours two types of changes developed. One was a homogenization process: with further disintegration of the Nissl structure, the cells were subjected to coagulation which finally led to ischemic changes of the ganglion cells associated with pyknosis. The second type of changes consisted in a vacuolization process which could affect the entire cell. In this case the cell acquired a foamy structure and in the end disintegrated completely or almost entirely so. As a rule, both processes were combined in one cell. Thus, for instance, the vacuoles developed usually in the periphery of the cell body, whereas the mass of the cytoplasm became homogeneous. At any time one could observe signs of shrinking cells. The protoplasm of the shrunken cell had a fine granular consistency, no longer revealing any traces of the Nissl structure. After the unfixed tissue had been kept at 37° C. for 48 hours, true incrustations of the ganglion cells were observed.

When death was preceded by a hypoxic phase of about 20 minutes' duration, initial swelling, though generally of a mild degree, likewise developed. It was succeeded by a homogenization process frequently associated with irregular transparencies in the protoplasm, which then developed a flaky appearance. Formation of larger vacuoles had stopped almost completely. The entire process developed within the first 3 hours, progressing but little over a period of 12 hours.

When the hypoxic phase lasted about 30 to 60 minutes, there existed, generally, from the very beginning, a certain pyknomorphia, as well as mild shrinkage. For as long as 12 hours the cell picture changed but little. Tigrolysis was occasionally more pronounced in the smaller pyramidal cells, whose protoplasm then developed a flaky structure.

Following a hypoxia lasting longer than about 60 minutes, the cells retained a structure corresponding to the equivalent picture for 30 minutes to 12 and 18 hours after death. Only occasionally did one of the large pyramidal cells show moderate shrinkage or mild swelling. Even after 48 hours the structure of the cells was maintained relatively well. The Nissl bodies showed merely a weaker response to staining, which

gave the cells a pale appearance. In general, the nuclei were only a little smaller than before, and darkened but little if at all. Even if a few putrefactive bacteria appeared in the vicinity of the cells, the cell structure was at first not affected. Only with stronger infiltration of the

FIGURE 9

Pyramidal cells of a cat exposed to severe hypoxia for 10 minutes. a, Swollen ganglion cells fixed 3 hours after death. b, Ganglion cells with swelling and flaky homogenization fixed 12 hours after death. The tissue has been kept at 37° C.

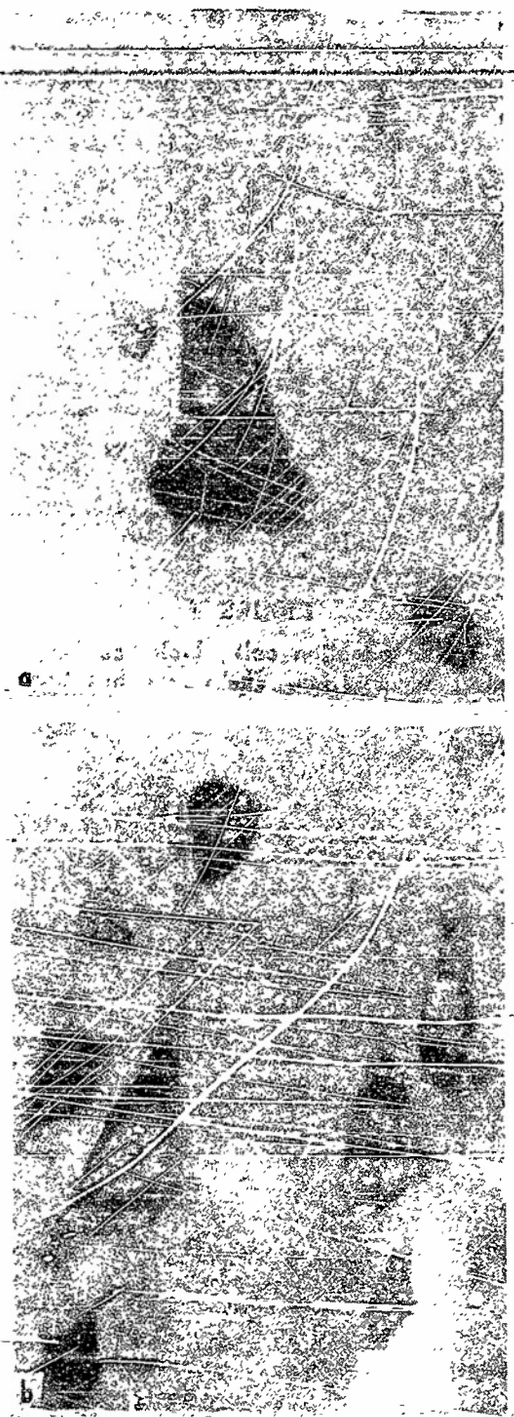

FIGURE 10

Pyramidal cells of a cat exposed to severe hypoxia for 35 minutes. a, Shrinkage of a ganglion cell. Not all Nissl bodies disappeared. b, Shrinkage and flaky homogenization of smaller pyramidal cells. The dendrites are traceable over a longer distance. The tissue has been kept at 37° C. and fixed 12 hours after death.

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NEUROHISTOLOGICAL INVESTIGATIONS ON GENERAL OXYGEN DEFICIENCY OF THE BRAIN - THE MORPHOLOGICAL BEHAVIOR OF THE GANGLION CELLS AFTER GENERALIZED ACUTE AND SUBACUTE HYPOXIA - PROJECT REPORT

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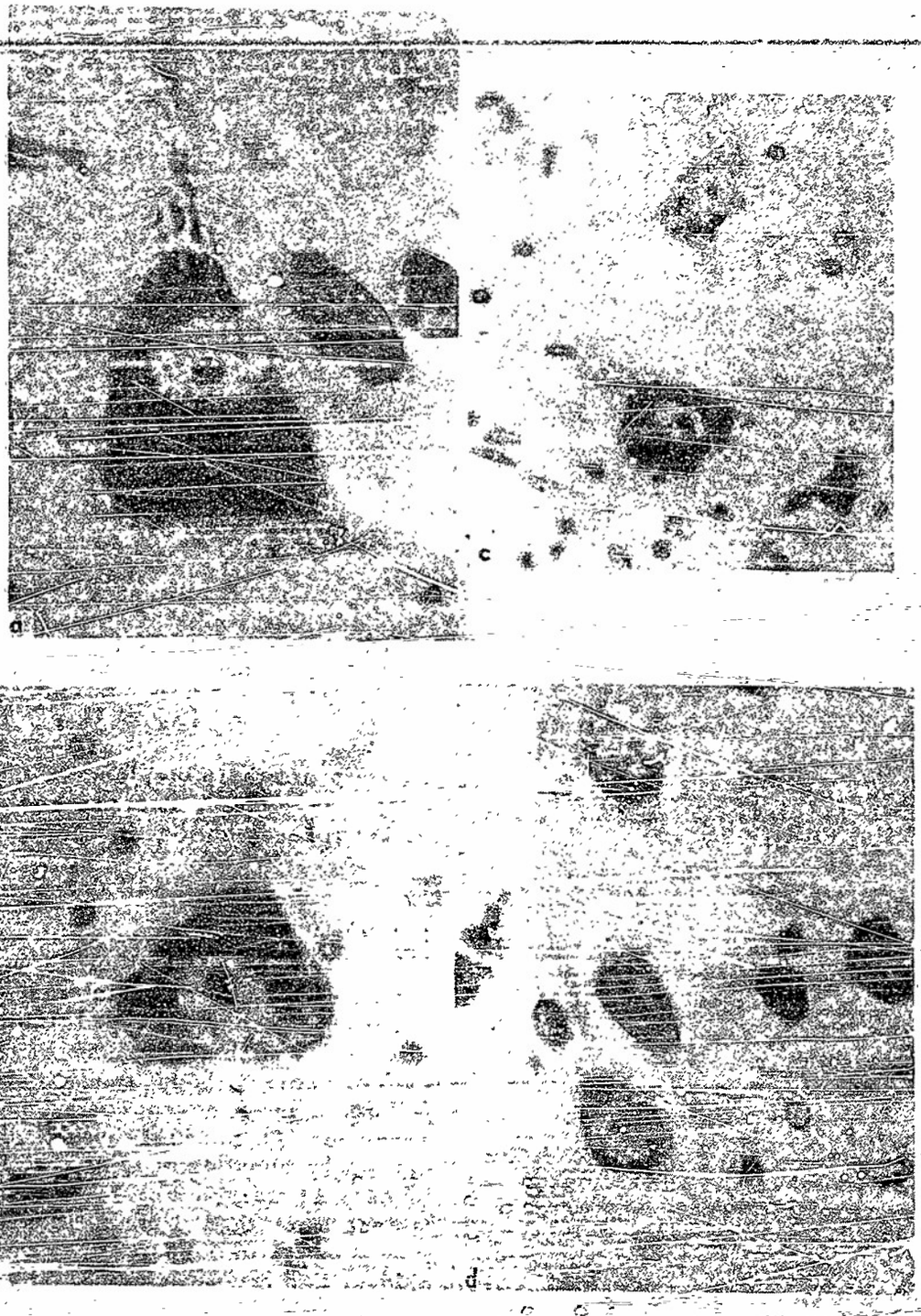
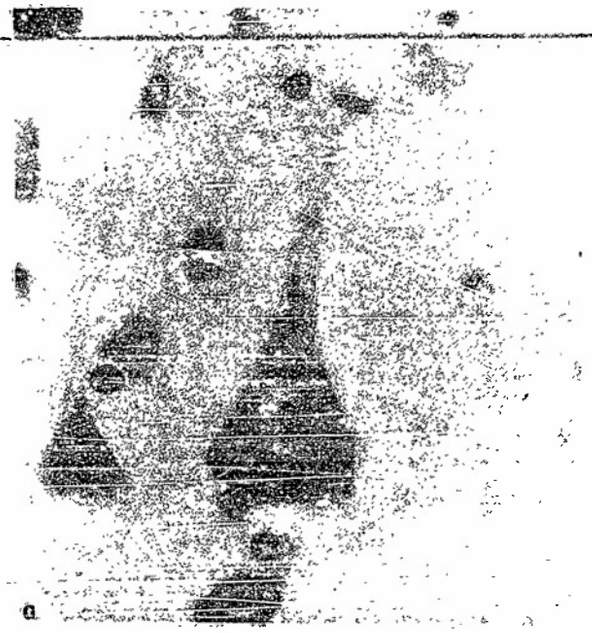


FIGURE 11

Four different cell types of a cat which had been exposed to severe hypoxia for 1½ hours. The tissue has been kept at 37° C. for 12 hours. a, Giant pyramidal cell of the motor cortex; b, thalamus cell surrounded by some putrefactive bacteria; c, pallidum cells; and d, cells of the second layer of the cortex (see fig. 1).

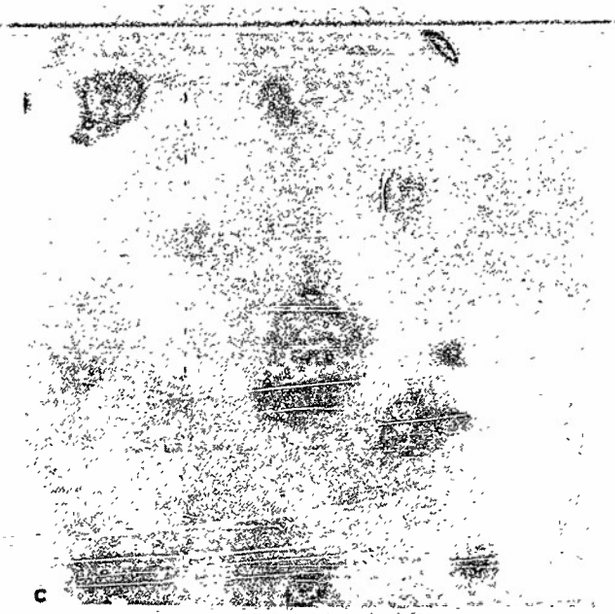


a

b

FIGURE 12

Ganglion cells of a cat which has been exposed to severe hypoxia for 3 hours. a, Pyramidal cell of the cortex; b, cell of the thalamus fixed 12 hours after death. The tissue has been kept at 37° C.



c

FIGURE 13

Smaller pyramidal cell of the same cat as in figure 12, fixed 48 hours after death. The unfixed tissue has been kept at 37° C.

bacteria did the protoplasm of the cells become dirty and blurred, and the nuclei smaller and darker (pseudo-homogenization).

HUMAN CASES

1. Methods

We examined a total of 45 brains of adults who had not died of an internal disease. In 30 cases death had occurred instantaneously or after a few minutes because of bleeding, suffocation, severe body trauma, or acute heart failure. In 4 cases death had followed an agony of 30 to 40 minutes' duration, being caused by bleeding or injurys to lungs and heart in case of trauma. For 11 cases the agony had lasted more than 1 hour, though never longer than about 6 to 7 hours. Two cases showed an unidentified traumatic edema of the larynx which led to slowly progressive suffocation. The others died of slow carbon monoxide poisoning, subacute alcoholic intoxication, increasing pulmonary edema with cardiac insufficiency following acute burns, and the consequences of bodily injuries.

All brains were fixed in formaldehyde. As far as possible we examined, in each case, sections

from various parts of the brain. After preliminary alcohol treatment, the blocks were embedded in cellordin or parlodion. Cresyl violet or thionine were used for staining the ganglion cells.

II. Findings

In the 30 cases of acute death, the findings were much the same. Shrinkage and vacuolization of the cells were the most frequent occurrence. In contrast to the early, localized pictures of shrinkage described in cats, a generalized cellular shrinkage was found in the human brains. In the cortex they involved the karyochrome cell types, the small and medium-sized pyramidal cells (figure 14a). The dendrites, especially the apical dendrite, were visible to a great extent and frequently were curled spiral-like (figure 14b). The larger cell types occasionally showed traces of the Nissl structure. Mostly, however, the protoplasm was dark, homogeneous, or contained very fine vacuoles. The nuclei were small and stained so deeply that they often could not be distinguished from the cell body. Vacuolization was found first of all in the small ganglion cells of the second and fourth layers. The picture designated by Nissl as *Wasserveraenderung* was very frequently encountered. The shrunken nucleus was surrounded by a halo whose outer limit was formed by a rim of homogeneous-looking protoplasm. The cell, as a rule, was markedly swollen. Phases of this alteration are demonstrated in figures 15a and 15b. The larger pyramidal cells could likewise undergo vacuolization, as shown in figure 15c. Shrinkage could predominate in one case; vacuolization, in the other. In general, both processes were intermingled. One could even observe shrinkage of the cell body and vacuolization of the dendrite in one and the same cell (figure 14b). Other cell types showed swelling associated with tigrolysis around the large, pale nucleus. In the cellular periphery, traces of the Nissl bodies could then still be visible. The least obvious changes were seen as a rule in the giant pyramidal cells and the large motor cells in the deeper parts of the brain stem. The Nissl bodies were generally easily detectable, because the unstained tracts were sometimes broadened. Some specimens showed a distinct tigrolysis or shrinkage. In some cases, homogenization of the cell body with shrinkage of the nucleus was prominent. In addition, there were pictures involving a rather flaky structure of the protoplasm, as seen in figure 9. In all these cases the time interval between death and fixation of the brain varied from 8 to 46 hours. Despite this large variance, we did not find any relationship between the severity of the changes and the span of time

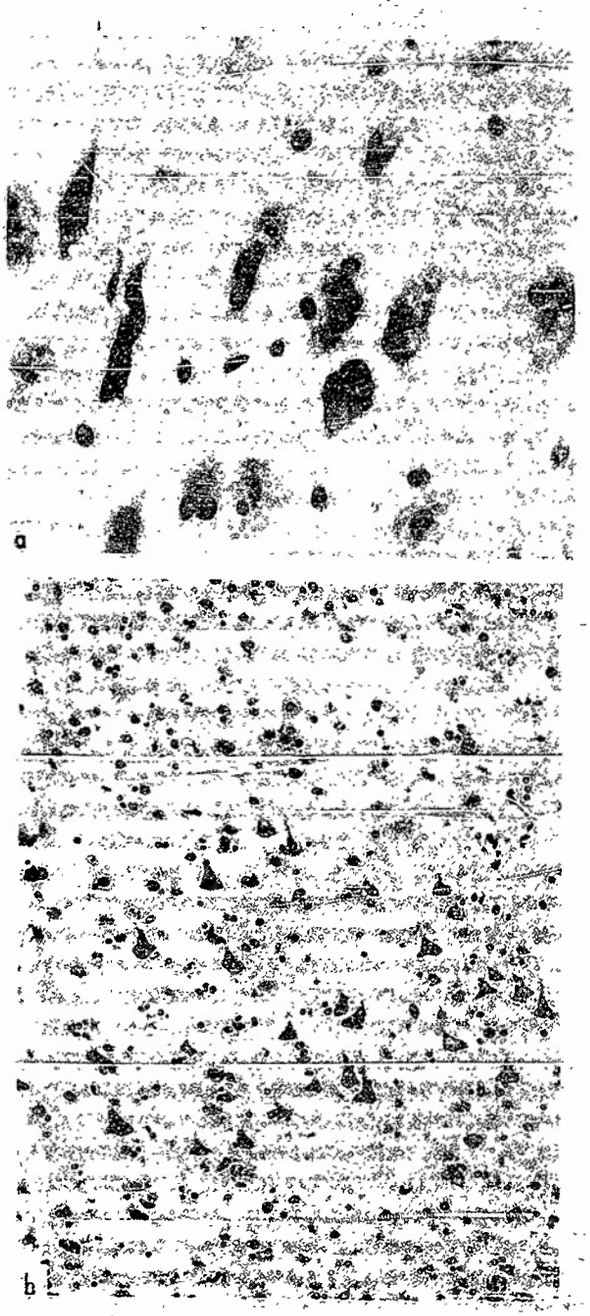


FIGURE 14

Shrinkage and homogenization combined with vacuolization of human ganglion cells in a case of acute suffocation. Fixation of the brain 18 hours after death.

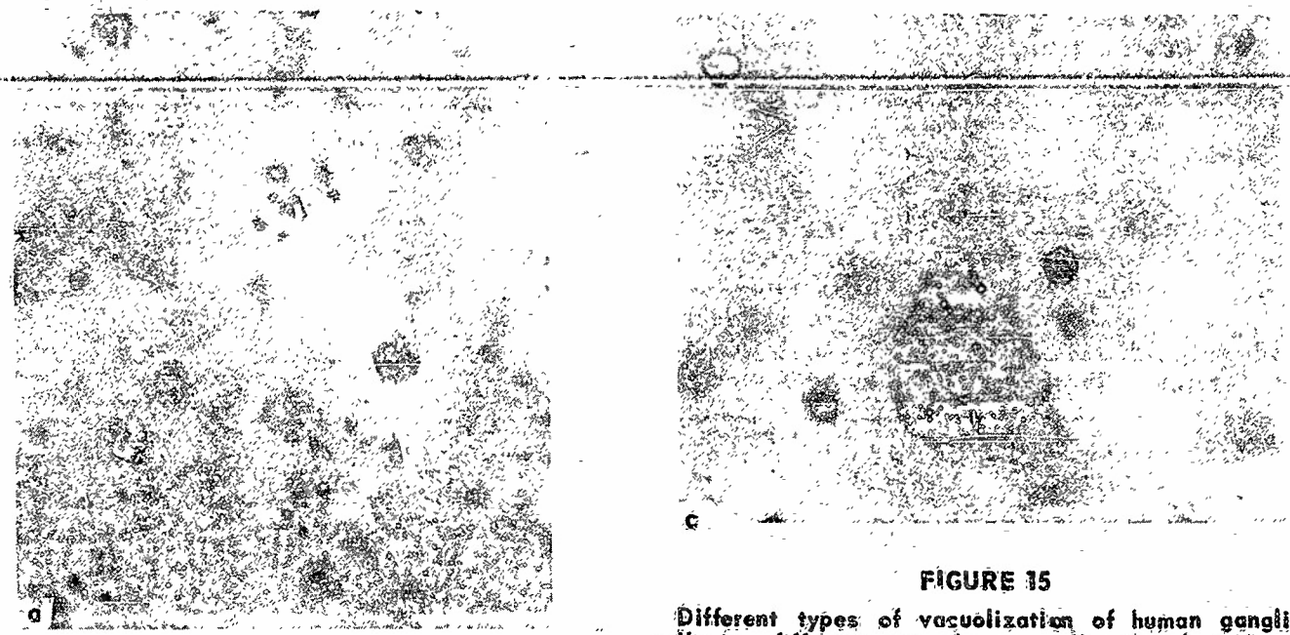
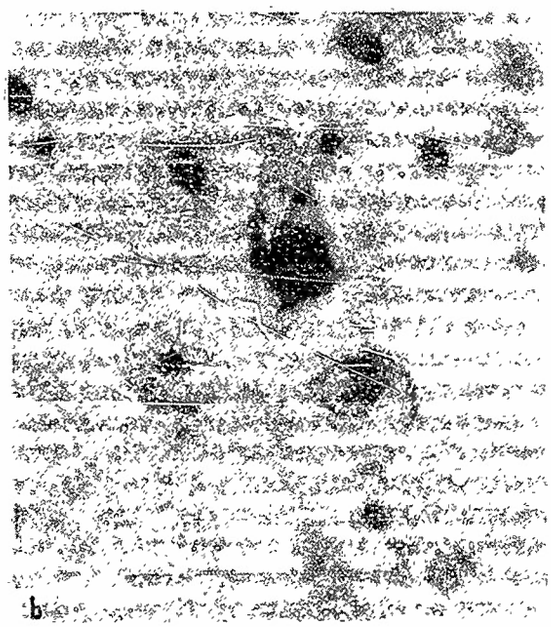


FIGURE 15

Different types of vacuolization of human ganglion cells in a fifferent case of acute suffocation from that in figure 14. Fixation of the brain 20 hours after death.



elapsing until the tissue was fixed. According to what we know from the experiments, the difference in the duration of the premortal hypoxia and the difference in the rate of cooling decide the prevalence of the one or the other type of cell change. Naturally, both factors cannot be determined accurately in human material. Also, the nutritive state of the cell at the instant of sudden death is most probably of some importance to the type of change which then develops. On the whole, however, it is no great concern whether vacuolization, homogenization, or shrinkage dominates the picture, since all these changes are definitely of the regressive type.

These changes differed noticeably from the findings we made in those cases in which the hypoxia lasted about 30 minutes and longer. Figures 16a and 16b show cells of such a case. They are very similar to the ganglion cells of a cat which had been exposed to severe hypoxia for 30 minutes (figure 10). Practically all cells showed, as a rule, mild shrinkage and well-defined dendrites. In most of such cases it was hard to decide whether to designate this picture as shrinkage or as pyknomorphia, the more so since the Nissl structure was present in many of the heavily stained cells. In other cells of the same size, however, a certain degree of homogenization already existed. The nuclei were usually small and dark. Vacuolization was

practically absent, even in the small cells of the second cortical layer. The giant pyramidal cells and the large somatochromes of the deeper brain stem primarily showed shrinkage combined with a varying degree of tigrolysis.

In the cases in which death followed an agonal hypoxia lasting not less than 1 nor longer than 6 or 7 hours, we found sometimes small, locally confined necrotic foci, which will be disregarded in this paper. The great majority of the cells showed neither vacuolization nor homogenization, neither shrinkage nor swelling. In all somatochromes the Nissl bodies were well formed; in the smaller cytochromes they had a dustlike structure. The nuclei were usually large and light, having a well-marked nucleolus and a delicate linin structure; sometimes they were slightly reduced in size and somewhat darker. In the larger pyramidal cells we observed occasionally that the Nissl bodies were separated a little more than usual by broadening of the unstained protoplasm tracts between them (figures 17a and 17b). Other cells were pyknomorphic. The cases showed hardly any difference in the behavior of the cells, although the time interval between death and fixation of the brain varied greatly, being 35 hours in one case, and the cooling of the corpse probably had been retarded in some cases.

III. Summary of the findings

In all cases without previous internal disease, in which death had occurred instantaneously or after a few minutes, and in which the time interval between death and fixation was not shorter than 8 hours, we found generalized changes in the ganglion cells, i.e., vacuolization and homogenization processes combined with shrinkage of varying degree. All the nuclei were shrunken and dark.

All cases in which death had occurred after an agonal hypoxia of about 30 minutes or longer, showed pyknomorphic cells or moderate shrinkage combined with beginning homogenization. The nuclei generally had undergone moderate shrinkage.

If death had been preceded by an agonal hypoxia of a duration ranging between at least 1 hour and 6 to 7 hours, the majority of the cells showed clear marking of the Nissl structure; there was neither shrinkage nor swelling. The nuclei were in general large and light, revealing a well-marked nucleolus and delicate linin struc-

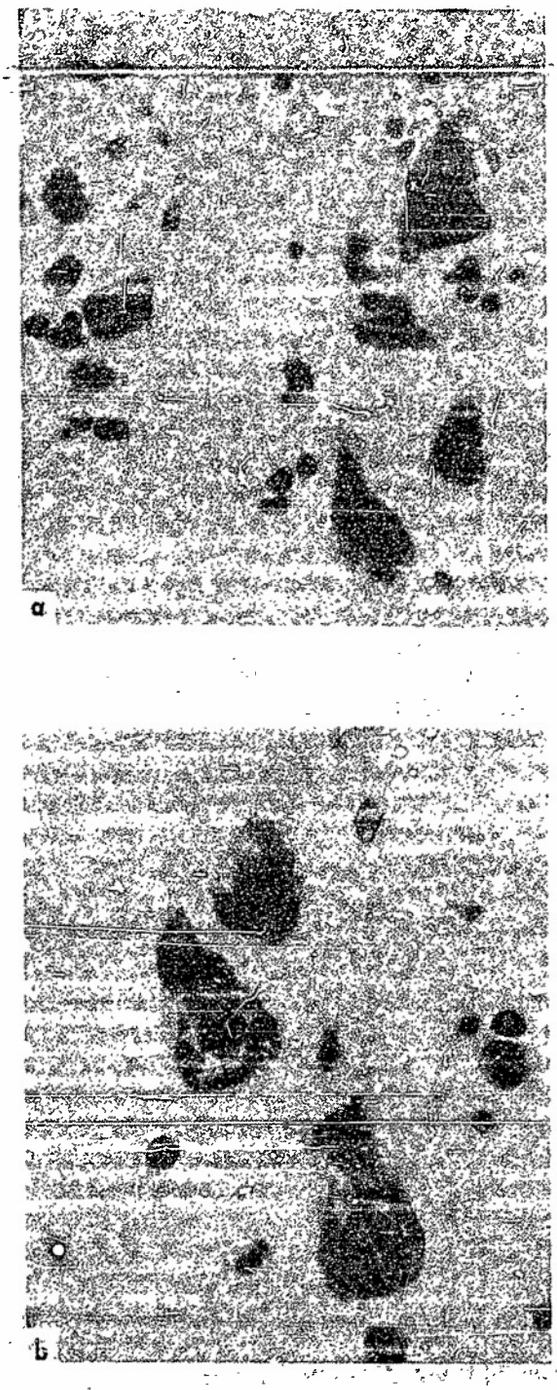


FIGURE 16

Ganglion cells of an individual who died after a severe hypoxia lasting for about 30 minutes (trauma). a, Flaky homogenization and dendrites traceable over a longer distance. b, Two smaller pyramidal cells still show some Nissl substance. Fixation of the brain 24 hours after death.

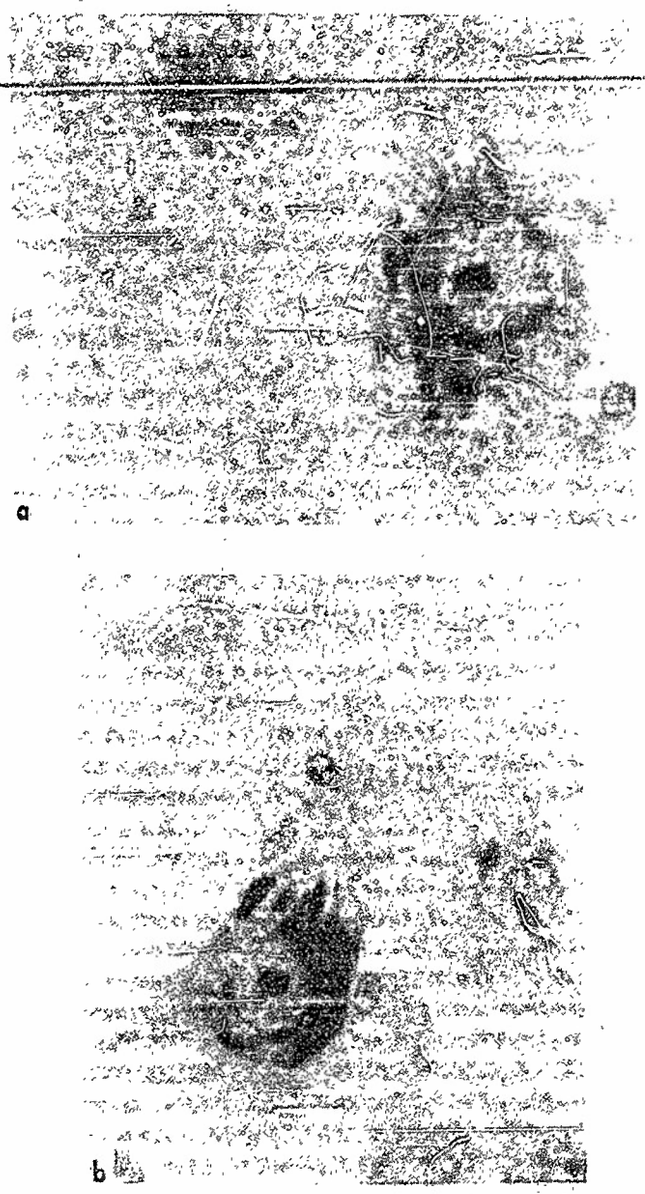


FIGURE 17

Ganglion cells of an individual who died after a severe hypoxia lasting for about 1½ hours (trauma). Fixation of the brain 24 hours after death.

ture, or they were darkened but slightly. Besides, we found pyknomorphia and separation of the Nissl bodies by slightly enlarged unstained protoplasm tracts. These pictures were observed even if the time span between death and fixation had been approximately 35 hours.

DISCUSSION

The findings in the cats are in essential agreement with those of man. Both series of investigations accentuate the primary importance of the duration of the severe hypoxia which precedes the anoxia of death.

The findings established in those individuals who died within a few minutes represent, so to speak, a snapshot from the film strip of a progressive disintegration process, whose time pattern could be observed more accurately in the cats. We shall not discuss in detail the nature of the various cytopathological pictures; it is sufficient for us to know that their development after death is the absolute rule. They are liable to be disposed of as "postmortal" or "autolytic" decomposition, which often presents an unpleasant secondary phenomenon to the histopathologist evaluating a histological preparation.

Ever since the first classification of pathological changes in ganglion cells was made, it has been known that, postmortally, the cells may undergo shrinkage and liquefaction processes associated with vacuolization, which greatly resemble the pictures of intravital cell disintegration. Even true karyorrhexis may develop post mortem, as has been found by Schmaus and Albrecht (7). Each textbook therefore cautions the reader against the misinterpretation of such postmortem alteration (cf. Weil (8); figure 14). On the other hand, we know that only a few hours after embolism distinct symptoms of disintegration develop in the focus and that the same holds for tissues excised from the organ. In the former case one speaks generally of necrosis or necrobiosis; in the latter, of autolysis. Could it possibly make any essential difference to the cell whose metabolism suddenly enters the anoxic state, whether this state is brought about by an embolism, by the cut of a knife, or by the failure of the central circulatory organ? It is our opinion that there is no difference whatsoever, and that the disintegration phenomena developing in the cell are principally the same in all three cases. If the complex of changes developing immediately after local vascular occlusion is called *necrobiosis*, there is no reason why this term should not be applied also to changes following acute cardiac standstill, as suggested by Rosenthal (9). Figures 18a and 18b demonstrate the cells of a 5-hour-old embolic softening which had been fixed

20 hours after death. Most of the cells show homogenization and vacuolization. It was impossible for us to distinguish them from the changes of corresponding stages in our cases of acute death, so great is the similarity of the morphological reactions. This similarity applies even to the postmortem development of the so-call'd incrustations of the Golgi apparatus, which are still believed to be especially typical of necrobiosis of intravital origin. More accurately, these changes develop with special regularity in the marginal sections of the foci of intravital origin, where a certain exchange of fluid with the blood-perfused adjacent area is still possible. In the brains of our cases kept in saline solution we observed these changes first in the cortical sections which were in direct contact with the solution. To give these facts due consideration and to avoid the terms of *postmortal alteration* or *autolysis*, which in pathology have acquired a flavor of factitiousness, it appears reasonable to apply the term *morphotropic mortal necrobiosis* also to ganglion cell changes after acute death.

Consequently, it is not true, as has been generally assumed (also by Nissl (2, 3)), that the state in which ganglion cells appear immediately after death can long be retained. As has been shown clearly by our experiments, this holds true only for those cases in which death has been preceded by a severe hypoxia for at least 1 hour. In such cases not even the cell structures changed, provided the unfixed brain had been kept in a sterile saline solution at 37° C. for 18 hours. With prolonged duration of such treatment there occurred gradual changes in the stainability but no essential alterations in the structural pattern. Naturally, in the end, these cells too will disintegrate somehow. This, however, occurs long after the postmortem phase which is of practical importance to the pathologist.

We assume that at the instant of the individual's death these cells, as individual elements, still possess a certain vital function which vanishes gradually without conceivable morphological criteria. In other words, we believe that the observed changes likewise represent a necrobiosis which, in contrast to the one discussed before, is at first not accompanied by noticeable structural changes. Analogous to our statements in regard to the astrocytes, the term *morphostatic mortal necrobiosis* can be applied in the present connection as well. Whenever a new term is introduced, one has to make sure that it is actually necessary and adequate and, moreover, that it helps to clear up an intricate problem. The objection may be raised, therefore, that for such cell pictures as are illustrated in figures 11a to 11d, Nissl has already coined the term *equivalent picture*. In this connection

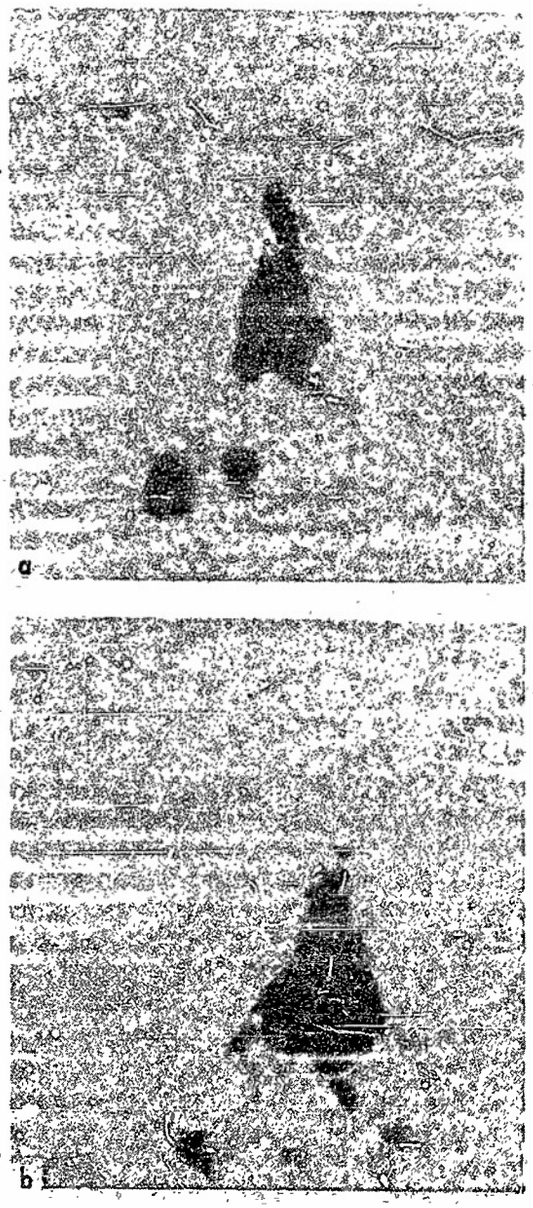


FIGURE 18

Ganglion cells from an embolic softening. The embolism occurred 5 hours prior to death. Homogenization (a) and vacuolization (b). Fixation of the brain 20 hours after death.

Spielmeyer (4), in the introduction to his book, says the following: "If under equal technical conditions identical pictures are obtained from normal tissue preparations, they may be regarded as equivalent to living tissue; all deviations from this equivalent preparation can be interpreted as pathological." In our opinion this conception is not quite correct. According to our present experience, a cell picture equivalent to that in the living tissue can be obtained only when the tissue is fixed during its normal activity. If in a case of instantaneous death—i.e., in virtually "normal" material—only a very short time is allowed to pass prior to fixation we will observe, instead of the equivalent picture, a beginning morphotropic necrobiosis, which undergoes progressive changes as the time interval between death and fixation is prolonged. Only when the tissue had been subjected to the pathological state of severe hypoxia for about an hour or longer prior to death did we find a picture which showed much greater similarity to the actual equivalent picture. Disregarding this similarity, this means that we have to deal with a morphological reaction during the anoxia of death, which is typical of a certain abnormal state of the cell during its life (subacute hypoxia): in other words, we have obtained a "positive" finding. We therefore believe we are justified in designating this reaction as *morphostatic mortal necrobiosis*. The high statistical frequency of this "pseudo-equivalent picture" of the cells in human autopsy material obviously induced Spielmeyer (4) to make the above statement, and is probably due to the fact that in many cases death follows only after prolonged agonal hypoxia.

When we attempt to make a morphological classification of a principally homogeneous process into distinct groups we naturally find that transitory stages exist. Even in the cases in which hypoxia had lasted about 20 minutes we observed a noticeable decrease in the incidence of vacuolization. Moreover, the predominant homogenization did not proceed so rapidly nor so uniformly as in the cases with shorter duration of the hypoxia. The cytoplasm, therefore, had a somewhat flaky appearance (figure 9b). The cells were in general slightly enlarged. However, since this alteration develops in the course of 12 hours, as seen from the experiment, it may be classified under morphotropic necrobiosis. In contrast hereto, the cases which had been exposed to hypoxia for about 30 to 60 minutes showed, from the very beginning, pyknomorphia of the cells as well as the pictures of moderate shrinkage. Only in part of the cells did a moderate homogenization occur after death. On the whole, however, the entire picture changes but little within 12 hours at 37° C. This consequently represents a transitory stage between the two types of necrosis. It is worth while mentioning that pyknomorphia and shrinkage had already developed toward the end of hypoxia, and that in the cases with longer duration of hypoxia—i.e., with the same stress on the cell—these changes subsided again when fixation was done immediately after death. Thus, they appear to be reversible when the cell is still under the same stress. Might this possibly be a morphological correlative to the process of accommodation known in altitude physiology, or in sports as "getting a second wind"? Not infrequently are different cell reactions found in one and the same brain. This is obviously due to the fact that the circulation during agonal hypoxia varies with the different brain sections, especially so in the presence of intracranial pressure.

It should be mentioned also that not all types of nerve cells respond equally fast to a sudden onset of anoxia. Among the cells in man, the giant pyramidal cells, the cells of the pallidum and substantia nigra, and the somatochrome cells of the pons and medulla show especially long resistance. In the cat such endurance is shown as a rule only by the last-mentioned somatochromes, whereas the giant pyramidal cells change relatively fast. In cases of morphostatic necrobiosis, on the contrary, these cells occasionally showed pronounced pyknomorphia. This caused the impression that they were lagging behind the cortical cells as far as their reaction is concerned. In such cases the large cells in the substantia reticularis of the medulla sometimes showed a picture similar to the fresh retrograde cellular alteration, associated with a displacement of the nucleus toward the cell wall. This probably represents a preliminary stage of changes which Buechner and Luft (10, 11, 12) found in the respective brain sections of guinea pigs exposed to prolonged hypoxia, and which might be connected with the overburdening of respiration and cardiac activity. The cells of the vegetative nuclei of the hypothalamus

showed, in our cases of acute death, no noticeable differences from the bulk of the cortical cells. (The nucleus supraopticus and the nucleus paraventricularis proved especially resistant to temporary hypoxia as shown by the experiments of Grenell and Kabat (13).

The findings of morphotropic necrobiosis, as reported above, are similar to the changes in the ganglion cells described by Lewis and Haymaker (14) in cases of high altitude death. In some of the cases described by the above-named authors, death had occurred within a few minutes; they found these changes in about half of their 58 cases. As to their statement, "It is of interest that the ganglion cell changes described could have occurred in so short a time," we can now say that these changes must have developed during the anoxia of death, i.e., post mortem, and not within the few minutes of increasing hypoxia. Camerer (15), performing autolytic experiments on human brains, found the same cell changes as we did in cases of acute death. In three-fifths of his 52 cases, death had occurred instantaneously or within a few minutes. He concluded that the pictures of ischemic cell changes are the regular end products of each "autolysis." Unless all of his cases had involved acute death after all, the only possible reason for his conclusion is, as he reported himself, a strong infiltration of bacteria in the brain sections he studied. Under the influence of a marked proliferation of bacteria, we too found a kind of pseude-homogenization. The high frequency of shrinkage in the pictures in his material might be explained by the fact that his brain sections were very small and, therefore in the unfixed state, easily bruised--the more so since he used scissors for the excision. In describing our cases, we mentioned briefly the incidence of shrinkage on account of pressure acting upon the unfixed tissue, as pointed out by Scharrer (6).

On the other hand, in reports on altitude chamber experiments with subacute hypoxia (Luft (11), Merk (16), Altmann and Schubothe (1), et al.), it is emphasized that no pathological changes could be detected except in local areas. In many cases fixation may have been accomplished too soon for changes to develop. Altmann and Schubothe (1) waited about two hours until they fixed the tissue; within this time some change should have appeared in cases of acut, death. The focal losses of cells and small softenings they described are in our opinion a secondary effect of hypoxia, caused by local functional disturbances of vessels or by nitrogen emboli, as described recently by Haymaker and Davison (17) in decompression deaths. This absence of widespread cell changes is now likewise comprehensible, and can no longer be interpreted as a "negative" finding.

Occasionally, one can observe a morphotropic necrobiosis of intravital origin within a morphotropic mortal necrobiosis. We examined one case, for instance, in which a head trauma had caused a local morphotropic necrosis. The victim survived the accident for 12 hours. Although respiration and circulation were in relatively good condition, he died very suddenly of respiratory paralysis. In the histological examination it was rather difficult to define the limit between the focus and its vicinity, since the changes observed in the focus were the same as, though less pronounced than, those found throughout the brain. Also, the astrocytes showed generalized clasmatodendrosis.

In this condition the question arises whether in such fresh local anoxic areas the necrobiotic disintegration of cells continues after death. After all, we cannot see any reason why this process in a local anoxic area should be interrupted by the superimposed anoxia of death. It will be merely retarded by the fall in brain temperature. Therefore we believe that the changes in the before-mentioned embolic softening were not 5 hours old, but 25 hours. They would have been even more pronounced had the brain retained its temperature until fixation. Furthermore, we are convinced that there is not only a morphostatic mortal necrobiosis but also a local morphostatic one of intravital origin. To this combination of changes probably belong some of the cases of apoplexy without pathological findings which have been observed from time to time. We examined such a case in which a stroke had occurred a short time before death. The autopsy showed thrombotic occlusion of an arteriosclerotic artery in the affected brain section. Histologically, however, no definite changes were found in the ganglion cells. In the affected areas there was observed a marked capillary stasis which was not present in the other parts of the brain. We are inclined to interpret this finding as a morphostatic thrombotic necrosis due to a gradually increasing occlusion of the vessel.

From our concept we may even make the following conclusion: if such a morphostatic focus had developed in a brain shortly before an acute death, we would find the paradoxical picture in which the cells within the focus are unchanged, and those outside the focus show signs of disintegration.

From all of this we learn that it is not generally appropriate to fix the brain as early as possible when acute circulatory disturbances occurred shortly before death. In case death followed a sufficiently long agony, the contrast between the focus and adjacent tissue becomes even greater when fixation is postponed for some time. In case of acute death it is advisable to fix the tissue as soon as possible in order to prevent a morphotropic change of the cells in the vicinity of the focus and, consequently, a blurring of the focal limits. In cases in which fresh tissue changes are anticipated, the type of death has to be considered more carefully than has been done previously.

How can we explain the fact that extremely severe changes in the cell structure occur after a sudden onset of general or local circulatory arrest, whereas they appear more and more seldom as the duration of a premortal hypoxia is increased? Obviously, this difference is based on biochemical processes. In cats that had breathed nitrogen for 2 to 3 minutes and had shown no electroencephalographic response for 20 to 30 seconds, Stone and collaborators (23) found an increase in lactic acid and inorganic phosphate combined with a decrease in phosphocreatine, whereas pyrophosphate and hexose phosphates had not yet changed. Following instantaneous death the freely fermentable sugar in the body-warm animal brain is broken down within the first 5 minutes, a great part of the glycogen within 15 minutes. The lactic acid production increases rapidly to its maximum within 25 to 30 minutes. (Jungmann and Kimmelstiel (18), McGinty and Gesell (19), et al.). About 30 minutes after such death, the phosphocreatine is hydrolyzed and part of the adenosine triphosphate broken down (20). Weil (8) correctly referred to this "explosive change of the cellular metabolism," concluding therefrom "that such chemical changes must result in physical changes of the structural make-up of a cell." We believe that all the changes which we described in cases of sudden death are morphologically related to these chemical processes, being caused primarily by the fact that upon circulatory standstill the decomposition products were left lying in and around the cell. It is particularly interesting that such essential chemical changes occurred after 30 minutes, the same time span after which we found the first distinct signs of swelling. When death is preceded by a critical hypoxic phase, the brain, obviously capable of utilizing lactic acid under normal conditions, discharges considerable quantities of it into the blood stream (21, 22). This happens even if hypoxia lasts no longer than 10 to 15 minutes. If death intervenes in such a circumstance, part of the lactic acid surplus has already been given off by the brain. Could this possibly be connected with our observation that, after a similarly short hypoxia, vacuolization was practically absent and a somewhat more flaky homogenization had appeared? We presume that this process, obviously quite complicated in detail and certainly not confined to lactic acid, will influence the morphological reaction of the cells. Loeschke's studies (22) indicate how important the degree of hypoxia is. Mild oxygen deficiency did not bring about a discharge but an inhibited utilization of lactic acid. It is also important that the oxygen-deficiency be kept on a constant level; as soon as recovery sets in, the lactic acid promptly returns to normal, and phosphocreatine is built up again (23).

No relevant data could be found in the literature concerning chemical processes developing during a hypoxia lasting 30 to 60 or more minutes. From the morphological reaction of the cells we are inclined to conclude that with prolonged hypoxia the over-all chemical condition of the cells will change as well. It may be that even the common pattern of cell metabolism will change after a certain duration of hypoxia. It seems that this problem opens up new avenues for a closer collaboration between biochemists and histopathologists, especially so when—aside from the Nissl picture, which was adequate for the present purpose—new staining methods are applied for the morphological analysis, as was done by Roizin (24).

SUMMARY

Experimental studies on cats, as well as examinations of human autopsy material in which

no organic disease had existed, showed that after death there is a great variation in the morphological behavior of the ganglion cells, depending on the duration of the severe hypoxia preceding death.

In cases of instantaneous death, the ganglion cells undergo changes which are principally the same as those in embolic softenings. They include the pictures of homogenization with and without shrinkage, vacuolization, and "incrustations of the Golgi apparatus." When the brain is kept at body temperature, a distinct "simple swelling" is noticed 30 minutes after death. Homogenization and vacuolization are well pronounced after 6 hours. This disintegration process is retarded as the temperature decreases.

If death was preceded by severe hypoxia lasting about 10 to 15 minutes, vacuolization hardly ever occurs and homogenization is incomplete and more flaky in appearance. If hypoxia lasted for about 30 minutes or longer, pyknomorphia and moderate shrinkage are found immediately after death, and change but little during the subsequent hours.

If hypoxia continued for about 60 minutes to several hours, the cells show no noticeable changes within the common time span between death and fixation. Their structure corresponds in general to the "equivalent picture." This structure, however, does not represent the equivalent picture of the living normal cell, but of a cell which had been in the pathological state of a severe hypoxia for at least 60 minutes.

The significance of these findings in relation to various neuropathological problems is discussed.

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